Inhibition of SLC drug transporter activities by environmental bisphenols

Bruyere, Arnaud ; Hubert, Céline ; Le Vee, Marc ; Chedik, Lisa ; Sayyed, Katia ; Stieger, Bruno ; Denizot, Claire ; Parmentier, Yannick ; Fardel, Olivier

Abstract: The plastic component bisphenol A (BPA) is suspected to exert deleterious effects towards human health and targets various cellular and molecular pathways, including activity of ATP-binding cassette drug transporters. The present study was designed to determine whether BPA and some derivatives, like its substitutes bisphenol F (BPF) and bisphenol S (BPS) and the flame retardant tetrabromobisphenol A (TBBPA), may additionally interact with solute carrier (SLC) drug transporters. Activities of the various following SLC transporters were inhibited in a major way (by >60%) by 100 M bisphenols: OCT1 and MATE1 (by BPA and TBBPA), OATP1B1 (by BPA, BPF and TBBPA), OATP1B3 and NTCP (by TBBPA) and OAT3 (by BPA, BPF, BPS and TBBPA); by contrast, activities of other transporters were not impacted (MATE2-K) or were stimulated (notably OCT1 by BPS and OCT2 by BPF). Transporter inhibitions due to bisphenols were concentrations-dependent, with half maximal inhibitory concentrations (IC50) ranging from 0.5 M to 73.5 M. BPA was finally shown to be not transported by OAT3, although inhibiting this transporter in a competitive manner. Taken together, these data indicate that bisphenols interact with SLC transporters, at concentration levels however rather higher than those occurring in humans in response to environmental exposure.

DOI: https://doi.org/10.1016/j.tiv.2016.12.009

Inhibition of SLC drug transporter activities by environmental bisphenols

Arnaud Bruyere[a], Céline Hubert[a], Marc Le Vee[a], Lisa Chedik[a,b], Bruno Stieger[c], Claire Denizot[d], Yannick Parmentier[d], Olivier Fardel[a,e,*]

[a]Institut de Recherches en Santé, Environnement et Travail (IRSET), UMR INSERM U1085, Faculté de Pharmacie, 2 Avenue du Pr Léon Bernard, 35043 Rennes, France

[b]Pole Pharmacie, Centre Hospitalier Universitaire, 2 rue Henri Le Guilloux, 35033 Rennes, France

[c]Department of Clinical Pharmacology and Toxicology, University Hospital Zurich, University of Zurich, Rämistrasse 100, 8091 Zurich, Switzerland

[d]Centre de Pharmacocinétique, Technologie Servier, 25–27 rue Eugène Vignat, 45000 Orléans, France

[e]Pôle Biologie, Centre Hospitalier Universitaire, 2 rue Henri Le Guilloux, 35033 Rennes, France

* Corresponding author at: Institut de Recherches en Santé, Environnement et Travail (IRSET), UMR INSERM U1085, Faculté de Pharmacie, 2 Avenue du Pr Léon Bernard, 35043 Rennes, France. Tel.: +33 2 23 23 48 80; fax: +33 2 23 23 47 94.

E-mail address: olivier.fardel@univ-rennes1.fr (O. Fardel).

Abstract

The plastic component bisphenol A (BPA) is suspected to exert deleterious effects towards human health and targets various cellular and molecular pathways, including activity of ATP-binding cassette drug transporters. The present study was designed to determine whether BPA and some derivatives, like its substitutes bisphenol F (BPF) and bisphenol S (BPS) and the flame retardant tetrabromobisphenol (TBBPA), may additionally interact with solute carrier (SLC) drug transporters. Activities of the various following SLC transporters were inhibited in a major way (by more than 60%) by 100 µM bisphenols: OCT1 and MATE1 (by BPA and TBBPA), OATP1B1 and OATP2B1 (by BPA, BPF and TBBPA), OATP1B3 and NTCP (by TBBPA) and OAT3 (by BPA, BPF, BPS and TBBPA); by contrast, activities of other transporters were not impacted (MATE2-K) or were stimulated (notably OCT1 by BPS and OCT2 by BPF). Transporter inhibitions due to bisphenols were concentrations-dependent, with half maximal inhibitory concentrations (IC_{50}) ranging from 0.52 µM to 73.5 µM. BPA was finally shown to be not transported by OAT3, although inhibiting this transporter in a competitive manner. Taken together, these data indicate that bisphenols interact with SLC transporters, at concentration levels however higher than those occurring in response to environmental exposure in humans.

Key words

Drug transporters; bisphenol A; bisphenol F; bisphenol S; tetrabromobisphenol; inhibition.

1. Introduction

Bisphenol A (BPA) is a synthetic chemical compound, largely used as a monomere in the production of polycarbonate plastics and epoxy resins, and by this way among the highest-volume chemicals produced worldwide. BPA is found in many consumer products, including food containers, paper products like thermal paper, water pipes and medical equipment (Halden, 2010). Humans are consequently widely exposed to this chemical, via dietary and non-dietary sources (Kang et al., 2006). Importantly, such an exposure is now thought to cause various deleterious effects for health (Rochester, 2013; Srivastava et al., 2015). BPA, through acting as a xeno-estrogen (Ben-Jonathan and Steinmetz, 1998), may thus exert reproductive and developmental toxicity (Golub et al., 2010; Peretz et al., 2014); it may also contribute to metabolic and cardiovascular diseases (Lang et al., 2008), may impair immunity (Jochmanova et al., 2015) and is suspected to have carcinogenic properties (Thompson et al., 2015). Such toxic effects have led to governmental restrictive regulations on the production and usage of BPA in North America and the European Union (Birnbaum et al., 2013). This also stimulates the development and production of alternative substances to replace BPA in a myriad of applications. Among such BPA substitutes, bisphenol analogues like bisphenol F (BPF) and bisphenol S (BPS) are major ones, even if they exhibited similar estrogenic and/or antiandrogenic activities than those of BPA (Chen et al., 2016). Endocrine-disrupting effects have additionally been reported for tetrabromobisphenol A (TBBPA), another bisphenol derivative, widely used as a brominated flame retardant in printed circuit boards and to which humans are also highly exposed (Birnbaum and Staskal, 2004).

Besides classical nuclear estrogen receptor alpha and beta, various molecular targets of BPA have been described, notably at the plasma membrane level. BPA thus interacts with ion channels and ATP-binding cassette (ABC) membrane transporters (Deutschmann et al., 2013; Soriano et al., 2016), suggesting that transport across plasma membrane may represent one of

the cellular process with which bisphenols interfere. In this context, it is noteworthy that main ABC drug efflux pumps have been shown to be targeted by bisphenols. Breast cancer resistance protein (BCRP/*ABCG2*) activity is thus inhibited by BPA and TBBPA; TBBPA additionally inhibits P-glycoprotein (P-gp/*ABCB1*) and multidrug resistance-associated protein (MRP) 1 (*ABCC1*) and MRP4 (*ABCC4*) (Dankers et al., 2013), whereas, by contrast, BPA stimulates P-gp activity (Jin and Audus, 2005), without however affecting P-gp expression (Peyre et al., 2014). Importantly, BPA, but not TBBPA, is transported by BCRP (Dankers et al., 2013); BPA is also a potential substrate for MRP2 (*ABCC2*) and MRP3 (*ABCC3*) (Mazur et al., 2012). These drug efflux pumps may therefore participate in the elimination of BPA and conjugated BPA metabolites, notably at the liver level (Moscovitz et al., 2016).

In addition to interacting with membrane ABC drug transporters, bisphenols may interfere with activity of solute carrier (SLC) drug transporters. The fact that BPA has been recently shown to inhibit activity of organic anion transporting polypeptide (Oatp/*Slco*) 1d1 (*Slco1d1*) in zebrafish fully supports this hypothesis (Popovic et al., 2014). Such potential interactions of bisphenols with SLC transporters remain however very poorly characterized, although SLC drug transporters represents key-actors of xenobiotic elimination pathway, notably in the liver and kidney where most of them primarily mediate drug uptake and whose inhibition may be the source of drug-drug interactions (Giacomini et al., 2010). The present study was therefore designed to analyze the possible interactions of various environmentally relevant bisphenols, *i.e.*, BPA, BPF, BPS and TBBPA, with activities of main human SLC drug transporters notably expressed in the liver, *i.e.*, organic cation transporter (OCT) 1 (SLC22A1), organic anion transporting polypeptide (OATP) 1B1 (SLCO1B1), OATP1B3 (SLCO1B3), OATP2B1 (SLCO2B1), sodium/taurocholate co-transporting polypeptide (NTCP/SLC10A1) and multidrug and toxin extrusion protein (MATE) 1 (SLC47A1), or in

the kidney, *i.e.,* OCT2 (SLC22A2), organic anion transporter (OAT) 1 (SLC22A6), OAT3 (SLC22A7) and MATE2-K (SLC47A2). Our data indicate that most of these SLC transporters constitute targets for bisphenols, especially for BPA and TBBPA.

2. Materials and Methods

2.1. Chemicals and reagents

BPA, BPF, BPS and TBBPA were provided by Sigma-Aldrich (Saint-Quentin Fallavier, France), as well as verapamil, probenecid, fluorescein, bromosulfophthalein (BSP) and amitriptyline. Chemical structures of bisphenols are shown in Fig. 1. Some of their basic molecular properties, including molecular weight, LogP predicted using the XLogP3 method, counts of hydrogen bond donors, hydrogen bond acceptors and rotatable bonds, and topological surface area, were given by PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and are indicated in Supplementary Table 1. [1-^{14}C]-Tetra-ethylammonium bromide (TEA) (sp. act. 3.5 mCi/mmol), [6, 7-^{3}H(N)] estrone-3-sulfate (E3S) (sp. act. 57.3 Ci/mmol), [^{3}H(G)]-taurocholic acid (sp. act. 5.0 Ci/mmol), P-[glycyl-1-^{14}C]-aminohippuric acid (PAH) (sp. act. 50.4 Ci/mmol) and [bis 2,6-^{3}H]-BPA (sp. act. 51.0 Ci/mmol) were from Perkin-Elmer (Boston, MA, USA). All other chemicals and reagents were commercial products of the highest purity available.

2.2. Cell culture

HEK293 cells overexpressing OCT1 (HEK-OCT1 cells), OCT2 (HEK-OCT2 cells), MATE1 (HEK-MATE1 cells), MATE2-K (HEK-MATE2-K cells) or NTCP (HEK-NTCP cells) and control HEK293 cells (HEK-MOCK cells), whose generation has already been described (Jouan et al., 2014; Le Vee et al., 2015), were routinely cultured in DMEM medium supplemented with 10% fetal calf serum (v/v), 100 IU/mL penicillin, 100 µg/mL

streptomycin, 1% (vol/vol) MEM non-essential amino acids solution (Life Technologies) and 1 µg/mL insulin. HEK293 cells overexpressing OAT1 (NM_004790) (HEK-OAT1 cells) or OAT3 (NM_004254) (HEK-OAT3 cells) were prepared by transduction of HEK293 cells by a lentiviral pLV-EF1-hOAT1-hPGK-GFP or pLV-EF1-hOAT3-hPGK-GFP vector, as previously reported (Jouan et al., 2014). Construction of the lentiviral vectors, production of lentivirus supernatants, transduction of HEK293 cells, cloning and initial characterization of HEK-OAT1 and HEK-OAT3 cells were performed by Vectalys (Labège, France). HEK-OAT1 and HEK-OAT3 were routinely cultured in DMEM medium as described above.

OATP1B1-, OATP1B3- and OATP2B1-transfected CHO cells were cultured in DMEM-low glucose containing 10 IU/mL penicillin, 10 µg/mL streptomycin, 10% (vol/vol) fetal calf serum, 50 µg/mL proline and 500 µg/mL G418, as previously reported (de Graaf et al., 2011).

Human differentiated hepatoma HepaRG cells (Gripon et al., 2002), which exhibit functional expression of OATPs (Le Vee et al., 2006), were routinely cultured in Williams' E medium (Life Technologies) supplemented with 10% (vol/vol) fetal calf serum, 100 IU/mL penicillin, 100 µg/mL streptomycin, 5 µg/mL insulin, 2 mM glutamine, and 5 x 10^{-5} M hydrocortisone hemisuccinate. Additional culture for two weeks in the same medium supplemented with 2% (vol/vol) dimethylsulfoxide was performed in order to get a full hepatocytic differentiation of the cells (Le Vee et al., 2006).

2.3. SLC transporter activity assays

The effects of bisphenols on SLC transporter activities were analyzed through determining intracellular accumulation of reference substrates of SLC transporters using a well-defined transport medium, as previously reported (Jigorel et al., 2005; Le Vee et al., 2015). The transport assay medium consisted of 5.3 mM KCl, 1.1 mM KH_2PO_4, 0.8 mM

$MgSO_4$, 1.8 mM $CaCl_2$, 11 mM D-glucose, 10 mM HEPES, and 136 mM NaCl; pH was adjusted to 7.4 value, except for the pH-sensitive MATE1 and MATE2-K transport assays for which pH was set at 8.4. Cells were first washed with transport assay buffer, then incubated at 37 °C for 5 min with transport assay buffer containing 40 µM [^{14}C]-TEA (used as a reference substrate for OCT1, OCT2, MATE1 and MATE2-K), 3.5 nM [^{3}H]-E3S (used as a reference substrate for OATP1B1, OATP2B1 and OAT3), 10 µM fluorescein (used as a reference substrate for OATP1B3), 43.4 nM [^{3}H] taurocholate (used as a reference substrate for NTCP) or 0.1 µM [^{14}C]-PAH (used as a reference substrate for OAT1), in the absence or presence of bisphenols or reference SLC transporter inhibitors. These reference inhibitors were 50 µM verapamil (for OCT1, MATE1 and MATE2-K), 100 µM BSP (for OATP1B1 and OATP2B1), 10 mM probenecid (for OATP1B3), 2 mM probenecid (for OAT1 and OAT3) and 100 µM amitriptyline (for OCT2). Reference inhibition of NTCP activity was done through removal of NaCl from transport assay medium and replacing it by 136 mM N-methyl glucamine (Jigorel et al., 2005). Cells were next washed twice with ice-cold phosphate-buffered saline (PBS), and finally lysed in distilled water. Intracellular accumulation of radiolabeled substrates was next measured by scintillation counting with a Beckman LS6500 (Beckman Coulter Inc, Fullerton, CA, USA). Intracellular accumulation of fluorescein was determined by spectrofluorimetry using a SpectraMax Gemini SX spectrofluorometer (Molecular Devices, Sunnyvale, CA); excitation and emission wavelengths were 485 and 535 nm, respectively. Values of substrate accumulation were normalized to total protein content, determined by the Bradford method (Bradford, 1976). Data were finally expressed as % of substrate accumulation in control cells not exposed to reference inhibitor or bisphenols; in this context, % of reduction of substrate accumulation was defined as 100% (accumulation in control cells) minus % of substrate accumulation in the presence of bisphenol or reference inhibitor, whereas % of stimulation of substrate accumulation corresponded to % of substrate

accumulation in the presence of bisphenol minus 100 % (accumulation in control cells). Data were also alternatively expressed as % of transporter activity found in control cells, arbitrarily set at 100%, according to the following equations:

For OATPs, OATs, OCTs and MATEs:

$$\% \text{ SLC transporter activity} = \quad \text{(A)}$$

with $Accumulation_{bisphenol}$ = substrate accumulation in the presence of bisphenol, $Accumulation_{control}$ = substrate accumulation in control cells and $Accumulation_{reference\ inhibitor}$ = substrate accumulation in the presence of a reference transporter inhibitor.

For NTCP:

$$\% \text{ NTCP activity} = \quad \text{(B)}$$

with $Accumulation_{Bisphenol}$ = taurocholate accumulation in the presence of bisphenol and sodium, $Accumulation_{Control/+Na^+}$ = taurocholate accumulation in control cells in the presence of sodium and $Accumulation_{Control/-Na^+}$ = taurocholate accumulation in control cells in the absence of sodium.

2.4. BPA accumulation assays

HEK-OAT3 cells and control HEK-MOCK cells were incubated at 37 °C for various lengths of time (from 1 to 10 min) with transport assay buffer containing 19.6 nM [^{14}C]-BPA. Cells were next washed twice with ice-cold PBS, and finally lysed in distilled water. Intracellular accumulation of BPA was then measured by scintillation counting as reported above.

2.5. Determination of kinetic parameters

For bisphenols inhibiting SLC drug transporter activity by at least 60 % when initially used at 100 µM, transport assays described above were performed in the presence of various

concentrations of bisphenols. Half maximal inhibitory concentrations (IC_{50}) were next determined using Prism 5.0 software (GraphPad Software, La Jolla, CA, USA) through nonlinear regression based on the following four-parameter logistic equation:

$$A= \qquad (C)$$

where A is the % of SLC transporter activity for a given concentration of bisphenol determined as described in equation (A) for OATPs, OATs, OCTs and MATEs and in equation (B) for NTCP, [I] is the bisphenol concentration in the medium, and Hill slope is a coefficient describing the steepness of the curve.

Kinetic parameters of E3S uptake into HEK-OAT3 cells (K_m and V_{max}) in the absence or presence of 50 μM BPA were estimated using Prism 5.0 software through nonlinear regression based on the Michaelis-Menten equation:

$$v= \qquad (D)$$

where v is the initial uptake rate of substrate, [S] is the substrate concentration in the medium, K_m is the Michaelis–Menten affinity constant, and V_{max} is the maximum uptake rate.

2.6. Statistical analysis

Experimental data were usually expressed as means ± SEM. They were statistically analyzed through analysis of variance (ANOVA) followed by the Dunnett's post-hoc test or through the F-test using the Prism 5.0 software. The criterion of significance was $p < 0.05$.

3. Results

3.1. Inhibition of organic cation transporter activity by bisphenols

Effects of BPA, BPF, BPS and TBBPA, each used at 100 μM, on TEA transport mediated by the organic cation transporters OCT1, OCT2, MATE1 or MATE2-K were determined. As shown in Fig. 2, BPA and TBBPA, like the reference inhibitor verapamil,

markedly inhibited uptake of TEA in HEK-OCT1 cells, by more than 80 % when compared to uptake in control conditions. BPF also significantly decreased TEA uptake in HEK-OCT1 cells, but in a more moderately manner (inhibition by 41.6 %), whereas, by contrast, BPS stimulated TEA accumulation by 61.9 % (Fig. 2). BPS, and also BPF, additionally stimulated TEA accumulation in HEK-OCT2 cells, whereas TBBPA reduced it and BPA was without effect (Fig. 2); the percentage of reduction of TEA uptake by TBBPA in HEK-OCT2 cells (39.4 %) was however lower than that caused by the reference OCT2 inhibitor amitriptyline (87.8 %). TEA uptake in HEK-MATE1 cells was inhibited by all bisphenols (Fig. 2); the percentages of these inhibitions by bisphenols ranged from 28.1 % (for BPF) to 66.8 % (for TBBPA) and were therefore lower than that caused by verapamil (98.0%) (Fig. 2). None of bisphenols impaired TEA accumulation in HEK-MATE2-K cells, in contrast to the reference inhibitor verapamil that markedly reduced it by 87.3 % (Fig. 2).

For inhibitions of TEA accumulation by 100 µM bisphenol reaching at least 60%, IC_{50} values towards SLC transporter activities were next determined. As shown in Fig. 3A, BPA IC_{50} towards OCT1 and MATE1 activities were 39.0 and 73.5 µM, respectively. With respect to TBBPA, IC_{50} values were 37.5 µM (for OCT1 activity) and 23.1 µM (for MATE1 activity) (Fig. 3B).

3.2. Inhibition of organic anion transporter activity by bisphenols

Effects of BPA, BPF, BPS and TBBPA, each used at 100 µM, on OATP-mediated uptake of reference substrates were first studied. As shown in Fig. 4A, BPA, BPF and TBPPA significantly decreased ES3 accumulation in CHO-OATP1B1 and CHO-OATP2B1 cells, in a rather notable manner; the percentages of accumulation decrease were thus comprised between 68.9 % (for down-modulation of E3S uptake by BPA in CHO-OATP1B1 cells) and 87.9 % (for down-modulation of E3S uptake by BPA in CHO-OATP2B1 cells). BPS also

reduced E3S uptake in HEK-OATP2B1 cells, but in a weaker manner (reduction by 43.6%); by contrast, this bisphenol failed to significantly alter E3S accumulation in HEK-OATP1B1 cells (Fig. 4A). BPS, in contrast to the reference inhibitor probenecid, did not also affect fluorescein uptake in CHO-OATP1B3 cells, whereas BPF significantly enhanced it by 38.2 % and BPA and TBBPA reduced it by 49.7 % and 73.8 %, respectively (Fig. 4A). Bisphenols were next shown to reduce E3S accumulation in hepatoma HepaRG cells, which constitutively display OATP activity (Le Vee et al., 2013); in this context, TBBPA was the most active, with an inhibitory effect on E3S uptake similar to that of the reference inhibitor BSP (Fig. 4B).

Effects of BPA, BPF, BPS and TBBPA, each used at 100 µM, on OAT- and NTCP-mediated uptake of reference substrates were next analyzed. As indicated in Fig. 5A, BPS moderately reduced PAH accumulation by 44.1% in HEK-OAT1 cells, whereas BPA stimulated it by 49.3% and BPF and TBBPA were without effect. All bisphenols markedly decreased E3S uptake in HEK-OAT3 cells, with percentage of inhibition ranging from 79.5 % (for BPF) to 94.1 % (for TBBPA) and therefore closed to the percentage of inhibition (97.2 %) caused by the reference inhibitor BSP (Fig. 5A). TBBPA was finally shown to markedly inhibit sodium-dependent accumulation of taurocholate in HEK-NTCP cells by 91.5 %, whereas BPA and BPF more modestly decreased it by 44.3 % and 39.2 %, respectively, and BPS was without effect (Fig. 5B).

For inhibitions of reference anionic substrate accumulation by 100 µM bisphenol reaching at least 60%, IC_{50} values towards SLC transporter activities were next determined. As shown in Fig. 6A, BPA IC_{50} values towards OATP1B1, OATP2B1 and OAT3 activities were around 9.2-26.4 µM. Those of BPF were 13.4 µM (for OATP1B1), 63.2 µM (for OATP2B1) and 26.8 µM (for OAT3) (Fig. 6B), whereas that of BPS toward OAT3 activity was 23.3 µM. IC_{50} values of TBBPA towards OATP1B1, OATP2B1 and OAT3 activities

were rather low (between 0.5 and 1 µM), whereas those towards NTCP and OATP1B3 activities were 4.5 µM and 12.1 µM, respectively (Fig. 7).

3.3. Characterization of OAT3-BPA interaction

Because OAT3 was the SLC transporter most potently inhibited by BPA (IC_{50}=9.17 µM; Fig. 6A), we next decided to characterize the nature of its interaction with this SLC transporter, primarily expressed by proximal renal cells (Burckhardt and Wolff, 2000). For this, we first analyzed the kinetic parameter (Km and Vmax) of E3S uptake by HEK-OAT3 cells in the absence or presence of 50 µM BPA through non-linear regression of E3S uptake in function of E3S concentration using the Michaelis-Menten equation. As indicated in Fig. 8, BPA was found to significantly enhance K_m without altering V_{max}, thus suggesting that it inhibited OAT3 activity by a competitive mechanism, possibly through interacting with the transport binding site of OAT3. To determine whether BPA may be a substrate for this SLC transporter, we next analyzed BPA uptake in HEK-OAT3 and HEK-MOCK cells. Results indicated that the two types of cells similarly accumulated BPA, whatever the incubation times with BPA (from 1 min to 10 min) (Fig. 9), thus discarding the hypothesis that BPA may be transported by OAT3. This conclusion is also supported by the fact that the OAT inhibitor probenecid used at 2 mM failed to alter BPA accumulation in HEK-OAT3 cells (data not shown). By contrast, HEK-OAT3 cells exhibited marked probenecid-inhibitable uptake of E3S when compared to HEK-MOCK cells (Supplementary Fig. 1), thus confirming that OAT3 was fully functional in HEK-OAT3 cells.

3.4. Prediction of potential drug-drug interactions due to inhibition of SLC transporters by BPA

We finally considered whether *in vitro* inhibition of SLC transporter activities by BPA may be relevant in term of drug-drug interactions (DDI). For this, we applied the criteria retained by the US Food and Drug Administration (FDA), according to which a potential DDI with respect to a drug inhibiting a transporter can be predicted from *in vitro* data when the maximum total plasma (bound plus unbound) concentration (C_{max}) of the investigated drug at steady state ([I]) divided by its *in vitro* inhibitory potency (IC_{50}) is greater than or equal to 0.1 ($[I]/IC_{50} \geq 0.1$) (Giacomini et al., 2010; Hillgren et al., 2013; Maeda and Sugiyama, 2013). For $[I_{BPA}]$, we retained the value of 6.5 nM, that corresponds to the unconjugated BPA C_{max} recently reported following oral administration of BPA to human volunteers (Thayer et al., 2015) and the value of 44 nM, that is the upper limit of the range of unconjugated BPA concentrations (2.2 nM to 44 nM) measured in blood samples from human populations (Vandenberg et al., 2010). None of these two BPA concentration values was predicted to cause potential DDI involving OCTs, MATEs, OATPs or OATs transporters, according to the FDA criteria (Table 1).

4. Discussion

The environmental contaminant BPA has previously been shown to interact with ABC drug efflux pumps like P-glycoprotein, MRP2 and BCRP. The data reported in the present study indicate that BPA and other bisphenol derivatives such as BPF, BPS and TBBPA can also inhibit various human SLC drug transporters, thus highlighting the fact that membrane drug transporters represent targets for environmental bisphenols.

BPA was thus demonstrated to inhibit activities of various SLC transporters, especially those of OCT1, MATE1, OATP1B1, OATP2B1 and OAT3 in transporter-overexpressing transfected cells; it concomitantly reduced constitutive OATP activity in hepatic HepaRG cells, indicating that its inhibitory effects towards SLC transporters were not

restricted to transfected cells. By contrast, BPA failed to impair activities of other SLC transporters such as OCT2, MATE2-K, OATP1B3 and OAT1, thus most likely discarding the hypothesis of a general and non-discriminating inhibitory effect of BPA towards membrane transporter activities. Inhibitory effects of BPA and other bisphenols towards some SLC drug transporters can rather be considered as specific, probably reflecting direct and transporter-dependent interactions of BPA and other bisphenols with substrate and/or regulatory binding sites on drug transporters, as classically described for drug transporter inhibitors (Montanari and Ecker, 2015). In this context, BPA appears to inhibit OAT3 in a competitive manner, thus suggesting that the bisphenol interacts with the substrate drug binding site of this transporter. However, BPA accumulation was similar in HEK-OAT3 and HEK-MOCK cells, indicating that BPA is not transported by OAT3, although interacting with the substrate drug binding. Non-competitive mechanism may also additionally account for inhibition of some transporters by BPA; the fact that BPA acts as an uncompetitive inhibitor of Oatp1d1 in Zebrafish supports this hypothesis (Popovic et al., 2014). In addition, it is noteworthy that BPA increased activity of OAT1. Such a cis-stimulation of SLC transporter activity has been already described for various chemicals (De Bruyn et al., 2013) and is also reported in the present study for other bisphenols such as BPF, that stimulated activity of OCT2 and OATP1B3, and BPS, that enhanced that of OCT1 and OCT2; in the same way, BPA has been previously shown to stimulate activity of the ABC efflux pump P-glycoprotein in placental cells (Jin and Audus, 2005). The molecular mechanisms that underlying such cis-stimulatory effects remain however yet very poorly characterized; they can reflect specific positive interactions with putative regulatory (or allosteric) binding sites on transporters, resulting in increased transport activity (Jin and Audus, 2005). With respect to SLC transporters, e.g. OATPs many of them have more than one binding site, which may mutually modulate each other : Hagenbuch and Stieger, Mol Asp Med 34: 396-412, 2014; Stieger and Hagenbuch,

Curr Top Membr 73: 205-232, 2014. Futures studies are therefore needed to more extensively characterize the exact mechanisms by which BPA differently interacts with some human SLC transporters.

Besides BPA, BPA substitutes like BPF and BPS and the brominated bisphenol TBBPA, used as flame retardant, also inhibited activities of SLC transporters. Among these bisphenols, TBBPA was the most potent inhibitor, acting towards the greatest number of transporters (activities of only OAT1 and MATE2-K were not impacted) and displaying low IC_{50} values, less than 1 µM for OATP1B1, OATP2B1 and OAT3. By contrast, OAT3 was the only transporter markedly inhibited by BPS, which thus appears as the less inhibitory bisphenol analyzed in the present study, while the inhibitory profile of BPF was relatively closed to that of BPA. Physico-chemical features differentially displayed by bisphenols may basically contribute to their various inhibitory effects towards SLC transporters. In this context, among basic molecular descriptors of bisphenols (Supplementary Table 1), hydrophobicity/LogP may be an important parameter to consider. Indeed, hydrophobicity has been reported to be one of key molecular descriptors for inhibition of various SLC transporters such as OAT3 (Duan et al., 2012), OCT1 (Ahlin et al., 2008) and OATP1B1 (De Bruyn et al., 2013), that are markedly inhibited by TBBPA, which, according to the calculated LogP value, is the most hydrophobic bisphenol (Supplementary Table 1). By contrast, hydrophobicity is not a molecular descriptor associated with blockage of OAT1 (Duan et al., 2012), which is not impacted by TBBPA. Further studies are however likely required to analyze quantitative structure-activity relationships for bisphenols-mediated inhibition of SLC transporters in a more detailed and accurate manner, knowing that a single molecular descriptor is not thought to be sufficient to appropriately describe binding to SLC transporters (Astorga et al., 2012).

The putative relevance of the SLC transporter inhibitions described in the present paper to human exposure to environmental bisphenols is probably a key-point that has to be considered. Inhibition of SLC transporters by BPA is very unlikely to cause DDI according to FDA criteria. This likely reflects the rather low plasma concentrations of BPA in humans, which are in the 1-44 nM range, owing to extensive intestinal and hepatic first-pass metabolism producing BPA-glucuronide (Thayer et al., 2015). In the same way, BPA-mediated SLC transporter inhibition may be presumed to have no consequence in terms of *in vivo* membrane transport of hormones like estrogens and thyroid hormones, which are notably handled by OATPs (Obaidat et al., 2012). A contribution of putative impairment of hormone transport in target cells or detoxifying organs like liver to known endocrine disruptive effects of BPA can therefore likely be discarded. With respect to TBBPA plasma concentrations in humans, they are either below the limit of quantification or very low, *i.e.*, less than 40 pM, reflecting rapid metabolism of TBBPA (Kibakaya et al., 2009; Schauer et al., 2006). Minimal concentrations of TBBPA (in the 0.5-1 µM range) required to inhibit SLC transporters can therefore not be reached *in vivo*, making very unlikely any DDI or perturbation of membrane transport of physiological substrates like hormones or bile acid due to TBBPA. Similarly, although plasma concentrations of BPF and BPS have not been yet characterized in humans, they may be hypothesized to be not sufficient to reach levels consistent with SLC transporter inhibition. It should however be kept in mind that humans are likely to be exposed not only to a single bisphenol, but to other contaminants and xenobiotics, that may also interact with drug transporters (Fardel et al., 2012), as recently demonstrated for notably organochlorine pesticides (Bucher et al., 2014), polychlorinated biphenyls (Nicklisch et al., 2016), diesel exhaust particle components (Le Vee et al., 2015) and perfluorinated surfactants (Nakagawa et al., 2009). Plasma levels of bisphenols in association with those of other pollutants, may therefore be sufficient to contribute to synergic or additive inhibitory effects towards drug

transporters, as already described for pesticide combinations (Pivcevic and Zaja, 2006). In addition, concentrations of bisphenols may be much higher in the gastro-intestinal tract (before first-pass metabolism) than in plasma, and SLC transporters notably expressed at the intestinal level, especially OATP2B1 and OCT1 (Giacomini et al., 2010), may therefore be hypothesized to be inhibitable by ingested bisphenols.

In summary, BPA and other environmental bisphenol derivatives, including TBBPA, were shown to down-modulate *in vitro* activity of various human SLC drug transporters. Such inhibitions of drug transporters occur however for relatively elevated concentrations of bisphenols (in the µM range), much higher than plasma concentrations observed in human populations (in the nM range for BPA and in the pM range for TBBPA). Such data make unlikely the fact that *in vivo* exposure to environmental bisphenols may lead to inhibition of SLC transporters in humans and, by this way to DDI or adverse effects, excepted for the gastrointestinal tract where sufficient concentrations of bisphenols may be reached.

References

Ahlin, G., Karlsson, J., Pedersen, J.M., Gustavsson, L., Larsson, R., Matsson, P., Norinder, U., Bergstrom, C.A., Artursson, P., 2008. Structural requirements for drug inhibition of the liver specific human organic cation transport protein 1. J Med Chem 51, 5932-5942.

Astorga, B., Ekins, S., Morales, M., Wright, S.H., 2012. Molecular determinants of ligand selectivity for the human multidrug and toxin extruder proteins MATE1 and MATE2-K. J Pharmacol Exp Ther 341, 743-755.

Ben-Jonathan, N., Steinmetz, R., 1998. Xenoestrogens: the emerging story of bisphenol a. Trends Endocrinol Metab 9, 124-128.

Birnbaum, L.S., Aungst, J., Schug, T.T., Goodman, J.L., 2013. Working together: research- and science-based regulation of BPA. Environ Health Perspect 121, A206-207.

Birnbaum, L.S., Staskal, D.F., 2004. Brominated flame retardants: cause for concern? Environ Health Perspect 112, 9-17.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72, 248-254.

Bucher, S., Le Vee, M., Jouan, E., Fardel, O., 2014. Regulation of hepatic drug transporter activity and expression by organochlorine pesticides. J Biochem Mol Toxicol 28, 119-128.

Burckhardt, G., Wolff, N.A., 2000. Structure of renal organic anion and cation transporters. Am J Physiol Renal Physiol 278, F853-866.

Chen, D., Kannan, K., Tan, H., Zheng, Z., Feng, Y.L., Wu, Y., Widelka, M., 2016. Bisphenol Analogues Other Than BPA: Environmental Occurrence, Human Exposure, and Toxicity-A Review. Environ Sci Technol 50, 5438-5453.

Dankers, A.C., Roelofs, M.J., Piersma, A.H., Sweep, F.C., Russel, F.G., van den Berg, M., van Duursen, M.B., Masereeuw, R., 2013. Endocrine disruptors differentially target ATP-binding cassette transporters in the blood-testis barrier and affect Leydig cell testosterone secretion in vitro. Toxicological sciences : an official journal of the Society of Toxicology 136, 382-391.

De Bruyn, T., van Westen, G.J., Ijzerman, A.P., Stieger, B., de Witte, P., Augustijns, P.F., Annaert, P.P., 2013. Structure-based identification of OATP1B1/3 inhibitors. Mol Pharmacol 83, 1257-1267.

de Graaf, W., Hausler, S., Heger, M., van Ginhoven, T.M., van Cappellen, G., Bennink, R.J., Kullak-Ublick, G.A., Hesselmann, R., van Gulik, T.M., Stieger, B., 2011. Transporters involved in the hepatic uptake of (99m)Tc-mebrofenin and indocyanine green. J Hepatol 54, 738-745.

Deutschmann, A., Hans, M., Meyer, R., Haberlein, H., Swandulla, D., 2013. Bisphenol A inhibits voltage-activated Ca(2+) channels in vitro: mechanisms and structural requirements. Mol Pharmacol 83, 501-511.

Duan, P., Li, S., Ai, N., Hu, L., Welsh, W.J., You, G., 2012. Potent inhibitors of human organic anion transporters 1 and 3 from clinical drug libraries: discovery and molecular characterization. Mol Pharm 9, 3340-3346.

Fardel, O., Kolasa, E., Le Vee, M., 2012. Environmental chemicals as substrates, inhibitors or inducers of drug transporters: implication for toxicokinetics, toxicity and pharmacokinetics. Expert Opin Drug Metab Toxicol 8, 29-46.

Giacomini, K.M., Huang, S.M., Tweedie, D.J., Benet, L.Z., Brouwer, K.L., Chu, X., Dahlin, A., Evers, R., Fischer, V., Hillgren, K.M., Hoffmaster, K.A., Ishikawa, T., Keppler, D., Kim, R.B., Lee, C.A., Niemi, M., Polli, J.W., Sugiyama, Y., Swaan, P.W., Ware, J.A., Wright, S.H., Yee, S.W., Zamek-Gliszczynski, M.J., Zhang, L., 2010. Membrane transporters in drug development. Nat Rev Drug Discov 9, 215-236.

Golub, M.S., Wu, K.L., Kaufman, F.L., Li, L.H., Moran-Messen, F., Zeise, L., Alexeeff, G.V., Donald, J.M., 2010. Bisphenol A: developmental toxicity from early prenatal exposure. Birth Defects Res B Dev Reprod Toxicol 89, 441-466.

Gripon, P., Rumin, S., Urban, S., Le Seyec, J., Glaise, D., Cannie, I., Guyomard, C., Lucas, J., Trepo, C., Guguen-Guillouzo, C., 2002. Infection of a human hepatoma cell line by hepatitis B virus. Proceedings of the National Academy of Sciences of the United States of America 99, 15655-15660.

Halden, R.U., 2010. Plastics and health risks. Annu Rev Public Health 31, 179-194.

Hillgren, K.M., Keppler, D., Zur, A.A., Giacomini, K.M., Stieger, B., Cass, C.E., Zhang, L., 2013. Emerging transporters of clinical importance: an update from the International Transporter Consortium. Clin Pharmacol Ther 94, 52-63.

Jigorel, E., Le Vee, M., Boursier-Neyret, C., Bertrand, M., Fardel, O., 2005. Functional expression of sinusoidal drug transporters in primary human and rat hepatocytes. Drug metabolism and disposition: the biological fate of chemicals 33, 1418-1422.

Jin, H., Audus, K.L., 2005. Effect of bisphenol A on drug efflux in BeWo, a human trophoblast-like cell line. Placenta 26 Suppl A, S96-S103.

Jochmanova, I., Lazurova, Z., Rudnay, M., Bacova, I., Marekova, M., Lazurova, I., 2015. Environmental estrogen bisphenol A and autoimmunity. Lupus 24, 392-399.

Jouan, E., Le Vee, M., Denizot, C., Da Violante, G., Fardel, O., 2014. The mitochondrial fluorescent dye rhodamine 123 is a high-affinity substrate for organic cation transporters (OCTs) 1 and 2. Fundam Clin Pharmacol 28, 65-77.

Kang, J.H., Kondo, F., Katayama, Y., 2006. Human exposure to bisphenol A. Toxicology 226, 79-89.

Kibakaya, E.C., Stephen, K., Whalen, M.M., 2009. Tetrabromobisphenol A has immunosuppressive effects on human natural killer cells. J Immunotoxicol 6, 285-292.

Lang, I.A., Galloway, T.S., Scarlett, A., Henley, W.E., Depledge, M., Wallace, R.B., Melzer, D., 2008. Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. JAMA 300, 1303-1310.

Le Vee, M., Jigorel, E., Glaise, D., Gripon, P., Guguen-Guillouzo, C., Fardel, O., 2006. Functional expression of sinusoidal and canalicular hepatic drug transporters in the differentiated human hepatoma HepaRG cell line. Eur J Pharm Sci 28, 109-117.

Le Vee, M., Jouan, E., Stieger, B., Lecureur, V., Fardel, O., 2015. Regulation of human hepatic drug transporter activity and expression by diesel exhaust particle extract. PLoS One 10, e0121232.

Le Vee, M., Noel, G., Jouan, E., Stieger, B., Fardel, O., 2013. Polarized expression of drug transporters in differentiated human hepatoma HepaRG cells. Toxicol In Vitro 27, 1979-1986.

Maeda, K., Sugiyama, Y., 2013. Transporter biology in drug approval: regulatory aspects. Mol Aspects Med 34, 711-718.

Mazur, C.S., Marchitti, S.A., Dimova, M., Kenneke, J.F., Lumen, A., Fisher, J., 2012. Human and rat ABC transporter efflux of bisphenol a and bisphenol a glucuronide: interspecies comparison and implications for pharmacokinetic assessment. Toxicological sciences : an official journal of the Society of Toxicology 128, 317-325.

Montanari, F., Ecker, G.F., 2015. Prediction of drug-ABC-transporter interaction--Recent advances and future challenges. Adv Drug Deliv Rev 86, 17-26.

Moscovitz, J.E., Nahar, M.S., Shalat, S.L., Slitt, A.L., Dolinoy, D.C., Aleksunes, L.M., 2016. Correlation between Conjugated Bisphenol A Concentrations and Efflux Transporter Expression in Human Fetal Livers. Drug Metab Dispos 44, 1061-1065.

Nakagawa, H., Terada, T., Harada, K.H., Hitomi, T., Inoue, K., Inui, K., Koizumi, A., 2009. Human organic anion transporter hOAT4 is a transporter of perfluorooctanoic acid. Basic Clin Pharmacol Toxicol 105, 136-138.

Nicklisch, S.C., Rees, S.D., McGrath, A.P., Gokirmak, T., Bonito, L.T., Vermeer, L.M., Cregger, C., Loewen, G., Sandin, S., Chang, G., Hamdoun, A., 2016. Global marine pollutants inhibit P-glycoprotein: Environmental levels, inhibitory effects, and cocrystal structure. Sci Adv 2, e1600001.

Obaidat, A., Roth, M., Hagenbuch, B., 2012. The expression and function of organic anion transporting polypeptides in normal tissues and in cancer. Annu Rev Pharmacol Toxicol 52, 135-151.

Peretz, J., Vrooman, L., Ricke, W.A., Hunt, P.A., Ehrlich, S., Hauser, R., Padmanabhan, V., Taylor, H.S., Swan, S.H., VandeVoort, C.A., Flaws, J.A., 2014. Bisphenol a and reproductive health: update of experimental and human evidence, 2007-2013. Environ Health Perspect 122, 775-786.

Peyre, L., Rouimi, P., de Sousa, G., Helies-Toussaint, C., Carre, B., Barcellini, S., Chagnon, M.C., Rahmani, R., 2014. Comparative study of bisphenol A and its analogue bisphenol S on human hepatic cells: a focus on their potential involvement in nonalcoholic fatty liver disease. Food Chem Toxicol 70, 9-18.

Pivcevic, B., Zaja, R., 2006. Pesticides and their binary combinations as P-glycoprotein inhibitors in NIH 3T3/MDR1 cells. Environ Toxicol Pharmacol 22, 268-276.

Popovic, M., Zaja, R., Fent, K., Smital, T., 2014. Interaction of environmental contaminants with zebrafish organic anion transporting polypeptide, Oatp1d1 (Slco1d1). Toxicol Appl Pharmacol 280, 149-158.

Rochester, J.R., 2013. Bisphenol A and human health: a review of the literature. Reprod Toxicol 42, 132-155.

Schauer, U.M., Volkel, W., Dekant, W., 2006. Toxicokinetics of tetrabromobisphenol a in humans and rats after oral administration. Toxicological sciences : an official journal of the Society of Toxicology 91, 49-58.

Soriano, S., Ripoll, C., Alonso-Magdalena, P., Fuentes, E., Quesada, I., Nadal, A., Martinez-Pinna, J., 2016. Effects of Bisphenol A on ion channels: Experimental evidence and molecular mechanisms. Steroids 111, 12-20.

Srivastava, S., Gupta, P., Chandolia, A., Alam, I., 2015. Bisphenol A: a threat to human health? J Environ Health 77, 20-26.

Thayer, K.A., Doerge, D.R., Hunt, D., Schurman, S.H., Twaddle, N.C., Churchwell, M.I., Garantziotis, S., Kissling, G.E., Easterling, M.R., Bucher, J.R., Birnbaum, L.S., 2015.

Pharmacokinetics of bisphenol A in humans following a single oral administration. Environ Int 83, 107-115.

Thompson, P.A., Khatami, M., Baglole, C.J., Sun, J., Harris, S.A., Moon, E.Y., Al-Mulla, F., Al-Temaimi, R., Brown, D.G., Colacci, A., Mondello, C., Raju, J., Ryan, E.P., Woodrick, J., Scovassi, A.I., Singh, N., Vaccari, M., Roy, R., Forte, S., Memeo, L., Salem, H.K., Amedei, A., Hamid, R.A., Lowe, L., Guarnieri, T., Bisson, W.H., 2015. Environmental immune disruptors, inflammation and cancer risk. Carcinogenesis 36 Suppl 1, S232-253.

Vandenberg, L.N., Chahoud, I., Heindel, J.J., Padmanabhan, V., Paumgartten, F.J., Schoenfelder, G., 2010. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Environ Health Perspect 118, 1055-1070.

Table 1: Prediction of potential drug-drug interactions (DDI) for BPA-mediated inhibitions of SLC drug transporters according to FDA criteria[a]

SLC transporter	Effect of BPA on transporter activity	Ratio $[I_{BPA}]/IC_{50}$		Prediction of potential DDI according to FDA criteria
		$[I_{BPA}]$=6.5 nM[b]	$[I_{BPA}]$=44 nM[c]	
OCT1	Inhibition (IC_{50}=39.0 µM)	0.00017	0.00111	No DDI
OCT2	No effect	ND[d]	ND	No DDI
MATE1	Inhibition (IC_{50}=73.5 µM)	0.00009	0.00060	No DDI
MATE2K	No effect	ND	ND	No DDI
OATP1B1	Inhibition (IC_{50}=20.4 µM)	0.00032	0.00216	No DDI
OATP1B3	No effect	ND	ND	No DDI
OATP2B1	Inhibition (IC_{50}=26.4 µM)	0.00025	0.00167	No DDI
OAT1	No effect	ND	ND	No DDI
OAT3	Inhibition (IC_{50}=9.2 µM)	0.00071	0.00478	No DDI

[a]FDA criteria: a potential clinical DDI may be predicted when $[I]/IC_{50} \geq 0.1$
[b]BPA C_{max} according to Thayer et al., 2015
[c]Upper limit of the range of BPA concentrations reported in the blood of human populations according to (Vandenberg et al., 2010)

[d]ND, not determinable

Legends to figures

Fig. 1. Chemical structures of bisphenols.

Fig. 2. Effects of bisphenols on OCT1, OCT2, MATE1 and MATE2-K activities.

OCT- and MATE-overexpressing HEK293 cell clones were incubated at 37°C for 5 min with the radiolabeled reference substrate TEA in the absence (untreated) or presence of reference inhibitors (50 µM verapamil for OCT1, MATE1 and MATE2-K and 100 µM amitriptyline for OCT2) or of bisphenols, each used at 100 µM. After washing, intracellular uptake of TEA was determined by scintillation counting and normalized to total cellular protein content. Data are the means ± SEM of at least three independent experiments. *, $p<0.05$ when compared to control untreated cells.

Fig. 3. Concentration-dependent effects of (A) BPA and (B) TBBPA towards OCT1 and MATE1 activities.

OCT1 and MATE1 activities were determined in the absence or presence of various concentrations of (A) BPA or (B) TBBPA, as described in Materials and Methods. Data are

expressed as percentages of transporter activity found in control untreated cells, arbitrarily set at 100%; they are the means ± SEM of three independent assays. IC_{50} values are indicated at the top of graphs.

Fig. 4. Effects of bisphenols on OATP activities.

(A) OATP-overexpressing CHO cell clones were incubated at 37°C for 5 min with reference substrates (radiolabeled E3S for OATP1B1 and OATP2B1 and fluorescein for OATP1B3) in the absence (untreated) or presence of reference OATP inhibitors (100 µM BSP for OATP1B1 and OATP2B1 and 2 mM probenecid for OATP1B3) or of bisphenols, each used at 100 µM. After washing, intracellular uptake of substrates was determined by scintillation counting (E3S) or spectrofluorimetry (OATP1B3) and normalized to total cellular protein content. Data are the means ± SEM of at least three independent experiments. *, $p<0.05$ when compared to control untreated cells. (B) HepaRG cells were incubated with E3S in the absence or presence of 100 µM BSP or of bisphenols, as described above. After washing, intracellular uptake of E3S was determined by scintillation counting and normalized to total cellular protein content. Data are the means ± SEM of three independent experiments. *, $p<0.05$ when compared to control untreated cells.

Fig. 5. Effects of bisphenols on OAT and NTCP activities.

(A) OAT-overexpressing HEK293 cell clones were incubated at 37°C for 5 min with radiolabeled reference substrates (PAH for OAT1 and E3S for OAT3) in the absence (untreated) or presence of the OAT inhibitor probenecid (2 mM) or of bisphenols, each used at 100 µM. (B) HEK-NTCP cells were incubated at 37°C for 5 min with taurocholate in sodium-containing medium in the absence (untreated) or presence of bisphenols, each used at 100 µM; reference inhibition of NTCP activity was done in parallel through removal of

sodium from transport assay medium. (A, B) After washing, intracellular uptake of substrates was determined by scintillation counting and normalized to total cellular protein content. Data are the means ± SEM of three independent experiments. *, $p<0.05$ when compared to control untreated cells.

Fig. 6. Concentration-dependent effects of (A) BPA and (B) (BPF) towards OATP1B1, OATP2B1 and OAT3 activities and of (C) BPS towards OAT3 activity.

Transporter activities were determined in the absence or presence of various concentrations of (A) BPA, (B) BPF and (C) BPS, as described in Materials and Methods. Data are expressed as percentages of transporter activity found in control untreated cells, arbitrarily set at 100%; they are the means ± SEM of three independent assays. IC_{50} values are indicated at the top of graphs.

Fig. 7. Concentration-dependent effects of TBBPA towards OATP1B1, OATP1B3, OATP2B1, OAT3 and NTCP activities.

Transporter activities were determined in the absence or presence of various concentrations of TBBPA, as described in Materials and Methods. Data are expressed as percentages of transporter activity found in control untreated cells, arbitrarily set at 100%; they are the means ± SEM of three independent assays. IC_{50} values are indicated at the top of graphs.

Fig. 8. Effects of BPA on kinetic parameters of E3S accumulation in HEK-OAT3 cells.

HEK-OAT3 cells were incubated with increasing concentrations of E3S in the absence or presence of 50 μM BPA for 5 min. E3S uptake velocity (V_{E3S}) was next fitted to [E3S] according to the Michaelis-Menten equation, in order to determine K_m and V_{max} values

(indicated at the top of the graph). Data are the mean ± S.E.M. of three independent experiments.*, $p<0.05$ when compared to control cells not exposed to BPA.

Fig. 9. Intracellular uptake of BPA in HEK-MOCK and HEK-OAT3 cells.

HEK-MOCK and HEK-OAT3 cells were incubated with radiolabeled BPA at 37°C for various length of times (from 1 min to 10 min). After washing, intracellular uptake of BPA was determined by scintillation counting and normalized to total cellular protein content. Data are the means ± SEM of three independent experiments. NS, not statistically significant.